CFAA—A FIXING AGENT FOR INSECT TISSUES [1,2]

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Some time ago, we developed an histological agent for use with insects which has proved to have wide utility. In particular, fixation is rapid, and so excessive hardening of the cuticle does not occur, thus facilitating preparation of sections with both exoskeleton and underlying tissues intact.

The original development of the reagent came about, because we wanted a fluid for field use with four characteristics: 1. very rapid killing power for large insects; 2. rapid penetration and fixation; 3. total evaporation to leave no stain; and 4. ready availability of constituents without licenses or permits. At the time, we were studying grasshoppers, and these insects when dropped into usual fixing or preserving agents, remain alive for some time and kick, thus damaging other specimens in the bottle. So we wanted death to be as rapid as possible. With such large objects being fixed whole and uncut in the field, penetrating power must be excellent. We wanted to use this fixing agent while traveling in an automobile or when stopped at motels. Therefore, we wanted no staining. Finally, for field use, we wanted to be able to get the materials easily without special permits or through special dealers.

Starting with a standard Formal-Acetic-Alcohol (FAA) mixture, various additions and changes were made. The final mixture, which

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we call CFAA, has chloroform as well as formalin, acetic acid, and alcohol. Originally it was developed using ethyl alcohol, which is excellent if available, with the following formula:

CFAA

Chloroform	100 ml.
Ethyl Alcohol (95%)	450 ml.
Formalin (40% Formaldehyde)	100 ml.
Water	350 ml.
Glacial Acetic Acid	20 ml.

Ethyl alcohol, however, outside of a laboratory is either expensive or, in out of the way places, difficult to obtain. So we changed to isopropyl alcohol, which is easily obtained.

IP CFAA

Chloroform	80 ml.
Isopropyl alcohol (100%)	450 ml.
Formalin	100 ml.
Water	350 ml.
Glacial Acetic Acid	20 ml.

The chloroform acts as a rapid killing agent and aids in penetration. The formulas have the maximum amount of chloroform that will remain stably mixed in solution. If the mixture separates because of addition of water from specimens or because of differential evaporation, one can add alcohol until the mixture is again homogeneous.

Fixation is rapid—even with large insects only ½-2 hours. Materials may remain in the mixture for extended periods (up to 4 weeks) without affecting later histological study, except for fatty tissues. However, it is best to transfer the fixed material from the CFAA within 6-8 hours to some preservative. We use 70% ethyl alcohol, or 30% isopropyl alcohol, or a mixture we call: 2-7-1, consisting of 20% isopropyl alcohol, 70% water and 10% glycerol. The last has some advantages in the field, for if the cover of the bottle is loosened, the preservative does not completely evaporate. With isopropyl alcohol, all of the reagents used in fixing and staining are obtainable on the open market.

Fixation in CFAA can be followed successfully by most routine histological or cytological techniques. With insects, particularly if they are left in the CFAA for a short time and then held in the 2-7-1 mixture, the exoskeleton remains supple. For dissection, the muscles and

nerves are particularly well preserved. Tissues stain well, with excellent detail. We have tried the CFAA—2-7-1 procedure also with a variety of tissues from rats—spleen, kidney, brain, muscle, liver, stomach, heart, lung, testes, etc.—and found it satisfactory. With pieces of relatively solid organs, about 1 cm. on a side, fixation is accomplished in 3-6 hours; fixing times over 24 hours are undesirable. Obviously, CFAA has no special value for mammalian studies unless the field-related properties are important. These tests were done, however, to check fixation of some well known "standard" material.

2.0108 CFAA—A fixing agent for insect tissues.

Abstract.—A mixture of chloroform (8 ml.), isopropyl alcohol (45 ml.), formalin (10 ml.), acetic acid (2 ml.) and water (35 ml.) is a rapid killing and fixing agent for tissues of insects. It leaves the cuticle easily managed and prepares insect specimens for easy dissection. It evaporates completely without staining, and all the materials are easily available. After fixation, specimens may be kept indefinitely in 30% isopropyl alcohol or in a mixture of isopropyl alcohol (20%), glycerol (10%) and water (70%).—Hubert and Mable Frings, *Department of Zoology, University of Oklahoma, Norman, Oklahoma* 73069.

Descriptors.—Histological techniques; preservation of animal specimens; fixation of tissues.